

---

## High and Low Plant Biodiversity: Two Strategies Encountered in Southwest Saskatchewan Prairie

Chantal Hamel<sup>1,2</sup>, Mike Schellenberg<sup>1</sup>, Juan Carlos Perez<sup>1,2,3</sup>, Keith Hanson<sup>1</sup>, and Richard Jeannotte<sup>4</sup>

---

<sup>1</sup>Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, SK S9H 3X2

<sup>2</sup>Department of Soil Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8

<sup>3</sup>Universidad Nacional de Colombia - AA 3840 Medellín

<sup>4</sup>Kansas State University, Division of Biology, Kansas Lipidomics Research Center  
506 Ackert Hall, Manhattan, KS, 66506 USA

**Key words:** Mixed grass prairie, restoration, soil lipids, plant community, soil-plant system, soil formation, soil biological activity, plant competition, crested wheatgrass, partitioning of biologically fixed nitrogen in legume and grass plant stands.

### Introduction

A large proportion of the American mixed grassland has been broken for annual cropping, mainly grains and oilseeds, or seeded with introduced grasses for forage production. Lesica and Deluca (1996) estimated that 6 – 10 million ha have been seeded to the introduced European cool season grass crested wheatgrass (*Agropyron cristatum*) in North America since the 1930's, when this plant was introduced to reduce soil erosion. Today, this plant species is still widely used to revegetate road sides and as a forage plant even though it was identified as an invasive species.

There is a growing interest in returning land from annual crop production to some form of perennial cover. This can be attributed in part to changing climate as well as changing economic realities that can make annual cropping unprofitable. In the past, European species have been recommended for forage production, but none of these species have good feed quality under the drought conditions characterizing the second part of the growing season in the mixed grassland ecozone. Recent research work has identified the native plant community as an interesting resource for cattle grazing (Jefferson et al. 2005).

During the 20<sup>th</sup> century, the area of the present mixed grass prairie ecozone had been under milder climatic conditions than before (Sauchyn et al. 2002). Models indicate that the immediate effect of global climate change would be to revert the Canadian prairie climate to conditions similar to those of the past where aridity periods could persist over a decade or longer. Nature has shown us that adaptation to aridity involves both the selection of adapted species within taxonomic groups and the selection of a functionally adapted community structure.

Our study was conducted in two experiments. In the first experiment, we wanted to describe the native mixed grass prairie systems to establish a baseline allowing the future measurement of climate change impact on the botanical composition and function of the native prairie in Grasslands National Park. We also wanted to gain information on the soil system during establishment of seeded native plant stands. Thus, we compared native plant-soil systems to systems recently restored into native vegetation. Finally, we wanted to increase knowledge on the ecology of crested wheatgrass. For this, we included

in the comparison stands of crested wheatgrass in the main experiment. In the second experiment, we tested the competitive ability of crested wheatgrass for the uptake of nitrogen biologically fixed by a neighboring legume.

## Methodology

### *First experiment*

We compared 4 sets (blocks) of 3 adjacent stands in Grasslands National Park: native, restored, and crested wheatgrass. The plant-soil systems were sampled in 2006 under conditions of good water availability in June and under drought, in August.

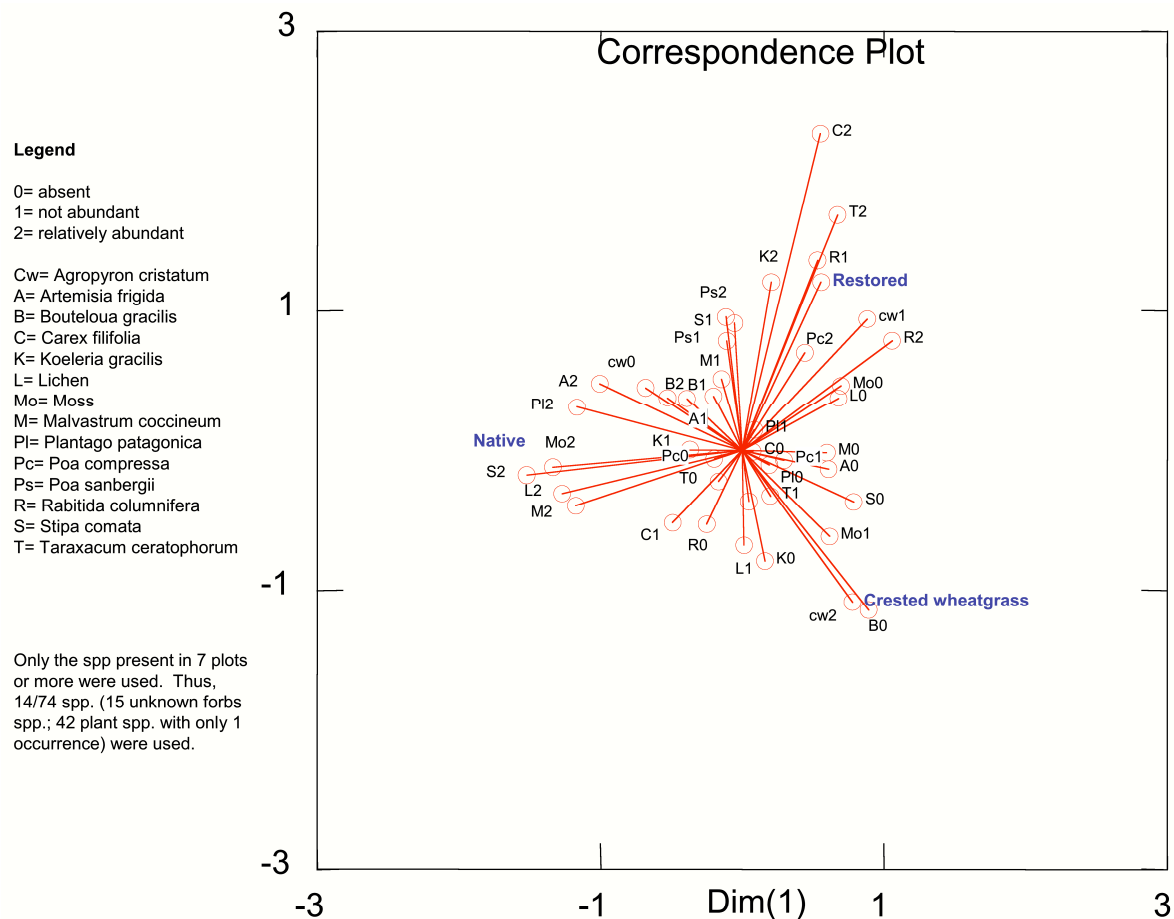
Plants from five 0.25 m<sup>2</sup> quadrats were identified, cut at soil level, and their total biomass determined after drying at 45°C. Plant species richness and the Simpson index of diversity were calculated (Legendre and Legendre 1998). The area of the soil occupied by the different species was also recorded. A composite sample of 15 cores was taken from each plot at each of two soil depths (0-10, 10-20) for the determination of gravimetric soil moisture, dehydrogenase activity (Casida et al. 1964), and soil organic matter quality through profiling of saturated n-alkanoic acids. The fresh weight equivalent of 4 g of oven dried soil was measured into glass centrifuge tubes, where 7.5 ml of dichloromethane:methanol (DCM:MeOH) mixture (1:2, v/v) was added followed by 2 ml of citrate buffer (i.e., 1:2:0.8 ratio). Tubes were capped with a Teflon lined caps and placed on a nutating shaker for 1 h followed by centrifugation at 3000 rpm for 10 min at ambient temperature. The supernatant was decanted into another centrifuge tube. The soil was then resuspended in the DCM:MeOH and citrate buffer and the extraction steps repeated. This was done for a total of 4 extraction cycles. Ten ml of DCM and 10 ml of citrate buffer was then added to the combined supernatants (for a final ratio of 2:2:1.8). This was centrifuged under the same conditions as above. The organic phase was taken with a pipet from the resulting layers, put into a vial and dried down under a flow of N<sub>2</sub>. The dried extracts were redissolved in 2 mL of chloroform:methanol (1:2, v/v) and stored in a freezer at -20°C. A 0.5 mL aliquot of each extracts was dried down under nitrogen flow and hydrolyzed using HCl/MeOH at a final concentration of 1.25 N during 4 hrs at 50°C. After hydrolysis, the mixtures were dried before adding 70 µL of pyridine and 30 µL MSTFA+1%TMCS, to silylate the hydroxy groups of the molecules through incubation at 50°C for 1 hr. Hexane (100 µL) was then added to each vial and mixtures were analyzed by GC-MS using a HP5ms column. One µL was injected in 1:5 split ratio and the mass spectrometer monitored ions from 50 to 650 m/z. Identification of the peaks was done using standards, database (NIST) and interpretation of the spectra.

### *Second experiment*

Five sets (blocks) of three plant species, crested wheatgrass, blue grama grass (*Bouteloua gracilis*), and the legume wild licorice (*Glycyrrhiza lepidota*), were sampled in June 2005, in one area of Grasslands National Park. Each plant was cut at soil surface level, dried at 45°C, ground, and analyzed for nitrogen concentration and <sup>15</sup>N:<sup>14</sup>N ratio by mass spectrometry. Plant content of nitrogen derived from biological fixation (from air) was calculated by the <sup>15</sup>N natural abundance method (Shearer and Kohl 1986).

### *Statistical analyses*

Plant biodiversity was analyzed by correspondence analysis (Legendre and Legendre 1998). Other data were analyzed by ANOVA after transformation to meet the requirement of normality when required. The significance of the difference between means was assessed using the least significant difference test at  $\alpha = 0.05$ .



**Figure 1.** Biplot of a correspondence analysis showing the relationship between plant stand type and plant community structure. The analysis was conducted on ranked data (0, species absent; 1, present in relatively low abundance; 2, present in relatively high abundance) included only the plant species present in seven of the 12 plots surveyed. Thus, only 14 of the 74 species identified on these sites were used in the analysis. Among the species excluded, 15 were unidentified forbs species and 42 species had a single occurrence.

## Results and Discussion

### Main experiment

Plant species richness was highest at the restored sites (Table 1). Simpson indices of diversity underscored the plant population sizes unevenness in restored stands as compared to the native sites (0.766 versus 0.824;  $P < 0.05$ ). A characteristic of the native sites was the extent of soil coverage, which was complete due to the mosses and lichens, colonizing the soil between plants. In other plant

stands, much of the soil between plants was left bare. In the restored stands, mosses and lichens probably had not yet colonized. Crested wheatgrass stands were characterized by greater proportions of bare soil. About 50% of the soil surface was left bare even though these stands were established over five decades ago (not shown). The only species able to use the space in between crested wheatgrass plants were mosses and lichens (Fig. 1) reflecting the ability of crested wheatgrass root system to aggressively occupy the top soil layer and utilize the limiting resource, in this case water. The mosses and lichens do not depend on soil resources. Crested wheatgrass biomass production was largest, but differences were not detected by the statistical analysis due to high variability in the data.

Dehydrogenase activity, a measure of potential biological activity, was highest under agricultural stands (Fig. 2a). The distribution of potential dehydrogenase activity in the soil profile varied with plant stands. Under agricultural plants, activity at 10-20 cm was 60% of that measured at 0-10 cm. In contrast, the activities at 10-20 cm under native and restored plant stands were 34% and 33% of the activity measured in the top soil layer. Reduced dehydrogenase under native and restored plant stands was associated with higher water availability (Fig. 2b), suggesting that water was not the factor limiting biological activity in these stands.

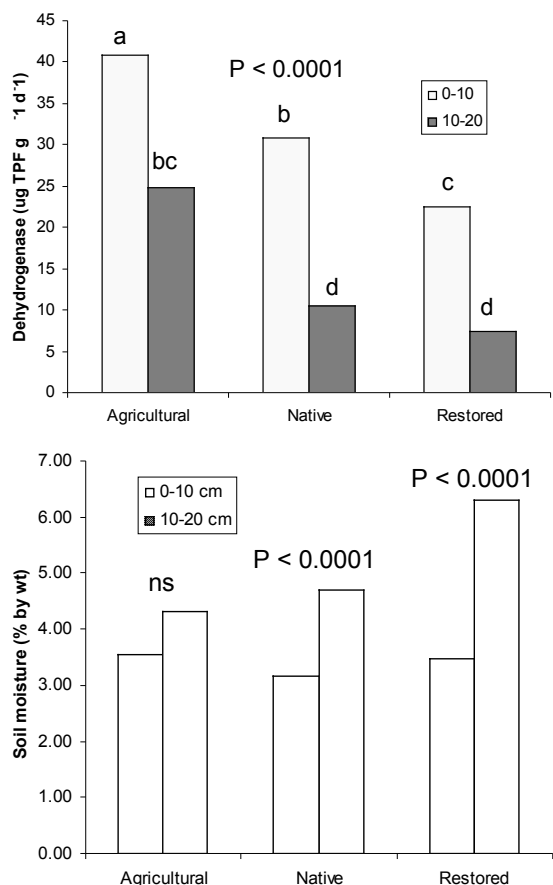


Figure 2. A) Average dehydrogenase activity and B) average soil moisture in agricultural plants, native and restored stands, on August 29-30. The Kruskal-Wallis test was used to test the effect of soil depth on soil moisture within plant stands, as the soil moisture data could not be normalized. Plant stand effect on dehydrogenase activity was assessed by ANOVA.  $N = 4$ , standard errors are in parenthesis.

Higher dehydrogenase activity in soil under the agricultural plant stands, which were almost pure stands of crested wheatgrass, is consistent with higher rate of carbon mineralization under this plant than under *Artemisia tridentata* Nutt., a native species (Chen and Stark, 2000). Higher dehydrogenase activity in the top soil layer under native and restored plant stands may be a result of higher soil temperature in these stands. Crested wheatgrass is a tall plant which produces a lot of shade and a thick layer of mulch that can protect the soil against temperature variations. Higher moisture in native stands soils is probably attributable to the mulching effect of mosses and lichens crusts. Higher moisture in restored soil might be explained by the high proportion of forbs in these stands. Forbs often have high water use efficiency.

Treatments significantly affected levels of 12:0, 18:0, 20:0, 21:0, 22:0, 23:0 n-alkanoic acids (Fig. 3). Also levels of some fatty acids were significantly different according to depth (11:0, 14:0, 20:0; not shown). A pattern of relative abundance of even over odd alkanolic acids was observed in all plant stands. This similarity in the profile from the different systems is unexplained, but may indicate fundamental effects of the environment on plant, faunal or microbial metabolisms. Sources of lipids in soils are: above and belowground plant biomass, soil

microorganisms and soil fauna. These factors should explain the discrepancies and similarities observed between the saturated n-alkanoic acids profiles of the different plant stands. The systems studied varied in the composition of their plant stands, thus pointing at microorganisms as the force evening the pattern of the different stands' alkanolic acid profiles. These alkanolic acids could come from fungi, higher plants suberin, cutin or waxes, or from insects. The abundance of these long chain alkanolic acids is lower in microorganisms than in plants. The composition of lipids from the different types of plants growing in each treatment remains to be studied in order to establish links between the plant lipids and the soil lipids, which most likely contain unaltered plant fatty acids.

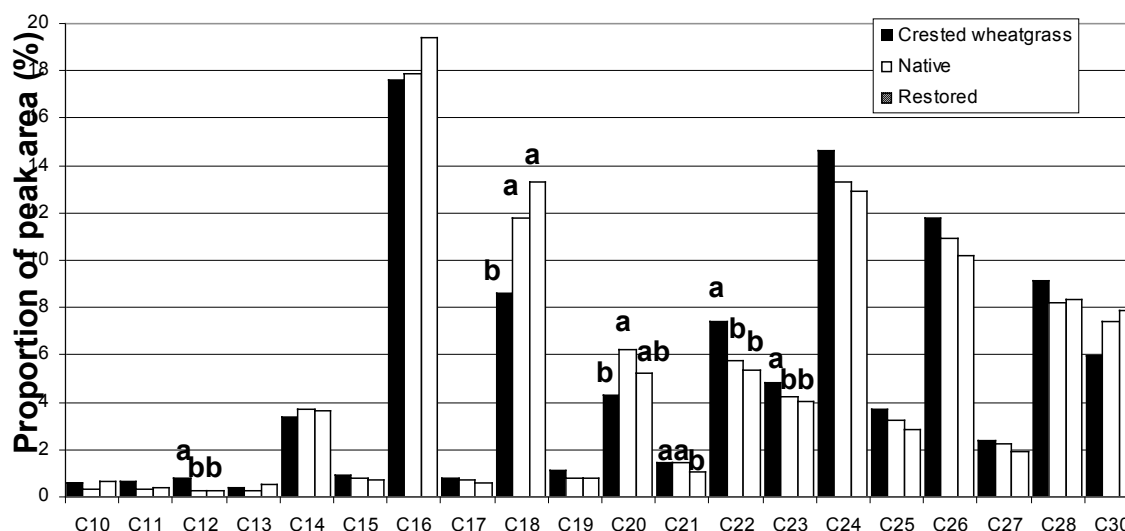


Figure 3. Profiles of saturated n-alkanoic acids in lipid extracts from the top 0-20 cm soil layer under crested wheatgrass, native and restored stands. Plant stand effect was assessed by ANOVA.  $N = 4$ . Bars associated with the same letter within a fatty acid are not significantly different according to LSD ( $P = 0.05$ ).

These preliminary results on conditions in different plant stands will be used to understand the soil microbial data that are still being collected. This data includes the description of soil microbial biomass and community structure, the abundance of key biotic components of the soil, such as fine roots, mycorrhizal fungi, and  $N_2$ -fixers.

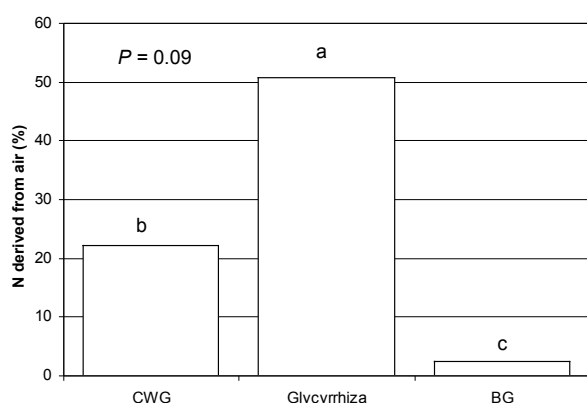


Figure 3. Nitrogen derived from the biological fixation activity of *G. lepidota* in the tissues of this legume (Glycyrrhiza), those of neighboring crested wheatgrass (CWG) and those of neighboring blue grama grass (BG), and the significance of the plant effect, as tested by ANOVA.  $N = 5$ . Bars associated with the same letter are not significantly different according to LSD ( $P = 0.05$ ).

### Second experiment

Due to high variability in the data, the effect of plant species on plants' nitrogen derived from biological fixation was significant at the 0.09 level only, according to ANOVA. About half of the nitrogen in *G. lepidota* was derived from the biological nitrogen fixation activity of this plant. The proportion of nitrogen derived from *G. lepidota* fixation was larger in crested wheatgrass than in blue grama grass, indicating the large competitive ability for nitrogen uptake of this invasive wheatgrass.

### Conclusion

We found that crested wheatgrass outcompetes other plant species at least through a large competitive ability for use of underground resources, in particular nitrogen from fixation by legumes. A few years after

seeding, plant stands had changed soil organic matter significantly, as indicated by saturated n-alkanoic acid profiling. A larger abundance of even over odd alkanoic acids, which was consistent in all plant stands' profiles, suggests that the nature of soil organic matter is largely influenced by an environmental factor impacting or selecting soil microbial metabolisms. More information on the soil microbial communities and soil organic matter composition are being gathered to establish a baseline for the evaluation of future climate change impacts on the botanical composition of plants in the mixed grass prairies.

#### **Reference cited**

- Casida, L.E., D.A. Klein and T. Santoro. 1964. Soil dehydrogenase activity. *Soil Sci. Soc. Amer. J.* 98:371-376.
- Chen, J. and J. M. Stark. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. *Soil Biology & Biochemistry* 32:47-57.
- Jefferson, P.G., A. D. Iwaasa, M. P. Schellenberg, and J. G. McLeod. 2005. Re-evaluation of native plant species for seeding and grazing by livestock on the semiarid prairie of western Canada. *Prairie Forum* 30:85-106.
- Legendre, P. and L. Legendre. 1998. *Numerical Ecology. Developments in Environmental Modelling* 20. Elsevier, Amsterdam. 853 p.
- Lesica, P., and T. Deluca. 1996. Long-term harmful effects of crested wheatgrass on Great Plains grassland ecosystems. *Journal of Soil and Water Conservation* 51:408-409.
- Sauchyn, D.J., E.M. Barrow, R.F. Hopkinson and P.R. Leavitt. 2002. Aridity on the Canadian Plains. *Géographie Physique et Quaternaire* 56:247-259.
- Shearer, G. and D.H. Kohl. 1986. N<sub>2</sub>-fixation in field settings: estimations based on natural <sup>15</sup>N abundance. *Aust. J. Plant Physiol.* 13:699-756.

**Table 1. List of plant species found in the main experimental sites of Grassland National Park.**

**Agricultural**

*Agropyron cristatum* (L.) Gaertn.  
*Agropyron smithii* Rydb.  
*Artemisia frigida* Willd.  
*Bouteloua gracilis* (BHK.) Lag.  
*Carex filifolia* Nutt.  
*Draba* spp.  
*Lepidium densiflorum* Schrad.

**Native**

*Agropyron intermedium* (Host) Beauv.  
*Antennaria parviflora* Nutt.  
*Artemisia frigida* Willd.  
*Bouteloua gracilis* (BHK.) Lag.  
*Carex filifolia* Nutt.  
*Crepis tectorum* L.  
*Eurotia lanata* (Pursh.) Moq.  
*Haplopappus spinulosus* (Pursh.) D.C.  
*Koeleria gracilis* Pers.  
*Lepidium densiflorum* Schrad.  
Lichen  
*Malvastrum coccineum* (Pursh) Gray  
Moss

**Restored**

*Achillea millefolium* L. var. *occidentalis* DC.  
*Adrosace septentrionalis*  
*Agropyron cristatum* (L.) Gaertn.  
*Agropyron dasystachyum* (Hook) Scribn..  
*Agropyron intermedium* (Hosti) Beauv.  
*Agropyron trachycaulum* (Link) Malte ex H. F. Lewis  
*Antennaria parviflora* Nutt.  
*Artemisia cana* Pursh  
*Artemisia frigida* Willd.  
*Astragalus bisulcatus* (Hook.) Gray  
*Astragalus pectinatus* Dougl.  
*Avena fatua* L.  
*Bouteloua gracilis* (HBK.) Lag.  
*Carex filifolia* Nutt.  
*Crepis tectorum* L.  
*Cruciferae* unidentified  
*Draba* spp.  
*Elymus subsecdus* (Link) A. & D. Love  
*Elymus trachycaulus* Link (Gould) ex Chinn.  
*Gallardia aristata* Pursh  
*Grindelia squarrosa* (Pursh) Dunal  
*Haplopappus spinulosus* (Pursh.) D.C.  
*Hordeum jubatum* L.  
*Koeleria gracilis* Pers.  
*Lepidium densiflorum* Schrad.  
Lichen  
*Lithospermum angustifolium* Michx.  
*Malvastrum coccineum* (Pursh) Gray  
Moss

Lichen  
*Malvastrum coccineum* (Pursh) Gray  
*Medicago sativa* L.  
Moss  
*Plantago patagonica* Jacq.  
*Poa compressa* L.  
*Taraxacum ceratophorum* (Ledeb.) D.C.

*Muhlenbergia cuspidata* (Torr.) Rydb.  
*Plantago patagonica* Jacq.  
*Poa compressa* L.  
*Poa sandbergii* Vasey  
*Psoralea argophylla* Pursh  
*Stipa comata* Trin. & Rupr.  
*Taraxacum ceratophorum* (Ledeb.) D.C.  
Unidentified  
unknown broomweed  
Unknown forb 4Dna  
Unknown forb 4DNb  
unknown rockcress  
unknown tiny

*Myosotis arvensis* (L.) Hill  
*Myosotis laxa* Lehn.  
*Plantago patagonica* Jacq.  
*Poa compressa* L.  
*Poa pratensis* L.  
*Poa sanbergii* Vasey  
*Polygonum aviculare* L.  
*Potentilla pennsylvanica* L. var. *atrovirens* (Rydb. Wolf)  
*Psoralea argophylla* Pursh  
*Petalostemon purpurea*  
*Rabtidia columnifera* (Nutt.) Woot. & Standl.  
*Solidago* spp.  
*Solidago* spp. 1  
*Solidago* spp. 2  
*Solidago* spp. 3  
*Sonchus* spp. Identification douteuse  
*Stipa comata* Trin. & Rupr.  
*Stipa curtiseta* (Hitchc.) Barkworth  
*Stipa viridula* Trin.  
*Taraxacum ceratophorum* (Ledeb.) D.C.  
*Tragopogon dubius* Scop.  
Unknown forb 3DR  
Unknown forb 4DRa  
Unknown forb 4DRb  
Unknown forb d2r1  
Unknown *Polygonum* sp.  
Vetch unidentified  
*Vicia americana* Muhl.